PB81-184780

Single-Breath Diffusing Capacity and Lung Volumes in Small Laboratory Mammals

(U.S.) National Inst. of Environmental Health Sciences, Research Triangle Park, NC

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Jun 80

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

MS-79-028

1. REPORT NO.	2.	3. RECIP
EPA-600/J-80-249	JOURNAL ARTICLE	PB81-184780

4. TITLE AND SUBTITLE	5. REPORT DATE
Single-breath Diffusing Capacity and Lung Volumes in Small Laboratory Mammals.	June 1980
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
J.Takezawa, F.J.Miller, and J.J.O'Neil	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
National Institute of Environmental Health Sciences Research Triangle Park, NC 27709	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research and Development (RTP,NC) Health Effects Research Lab US Environmental Protection Agency Research Triangle Park,NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Journal of Applied Physiology, 48(6):1052-1059, 1980

16. ABSTRACT

We measured the single-breath diffusing capacity for carbon monoxide (DLco), total lung capacity(TLC), functional residue capacity(FRC), and residual volume(RV) in anesthetized male hamsters, rats, guinea pigs, and rabbits whose weights varied from 40 to 3,500g. TLC (defined as an airway pressure of 25 cmH_2O) was calculated by neon dilution. The DLco was estimated by a modification of the single-breath method. There was a high correlation between body weight and our measurement of both the diffusing capacity and the lung volumes. No significant difference in DLco was observed in rats when measured in different body positions, at airway pressures of 10 or 20 cmH_2O, from FRC or RV, in male or female rats, or following hyperventilation.

PRODUCT OF:
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Small animal pulmonary function Comparative physiology Hamster, Rabbit, Guinea pig, and Rat		06T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA 600/J-80-249
Journal Article

Single-breath diffusing capacity and lung volumes in small laboratory mammals

JUN TAKEZAWA, FRED J. MILLER, AND JOHN J. O'NEIL
The National Institute of Environmental Health Sciences, Research Triangle Park 27709; and Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711

TAKEZAWA, JUN, FRED J. MILLER, AND JOHN J. O'NEIL. *Single-breath diffusing capacity and lung volumes in small laboratory mammals.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 48(6): 1052–1059, 1980.—We measured the single-breath diffusing capacity for carbon monoxide (D_{LCO}), total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV) in anesthetized male hamsters, rats, guinea pigs, and rabbits whose weights varied from 40 to 3,500 g. TLC (defined as an airway pressure of 25 cmH_2O) was calculated by neon dilution. The D_{LCO} was estimated by a modification of the single-breath method. There was a high correlation between body weight and our measurement of both the diffusing capacity and the lung volumes. No significant difference in D_{LCO} was observed in rats when measured in different body positions, at airway pressures of 10 or 20 cmH_2O, from FRC or RV, in male or female rats, or following hyperventilation.

small animal pulmonary function; comparative physiology; hamster; rat; guinea pig; rabbit

EVALUATION OF PULMONARY FUNCTION in small laboratory mammals may be valuable in studying growth and development of normal lung and in evaluating changes associated with exposure to environmental pollutants or pulmonary toxicants. Because facilities for exposure to environmental pollutants are limited and expensive, the use of small animals is advantageous. We have measured the single-breath diffusing capacity for carbon monoxide (D_{LCO}), total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV) in normal male hamsters, rats, guinea pigs, and rabbits whose weights varied from 40 to 3,500 g. In addition, we have compared the results of these measurements when made in male and female rats, in different body positions, from FRC or RV, to different airway pressures, and following hyperventilation. There is a high correlation between body weight and both the diffusing capacity and the lung volumes.

MATERIALS AND METHODS

Animals. Male animals (Charles River Laboratories, Wilmington, MA) were anesthetized by an intraperitoneal injection of pentobarbital sodium (Nembutal, Abbott Laboratories, Waukegan, IL) as follows: Golden Syrian hamsters (40–120 g) and Sprague-Dawley rats (50–400 g), 50 mg/kg; and Hartley guinea pigs (250–1,000 g), 25 mg/kg. New Zealand White rabbits (1,300–3,500 g, Dutchland Laboratory Animals, Denver, PA) were anesthetized by a dose of approximately 100 mg/kg injected via an ear vein. Each animal was tracheostomized and an appropriately sized Luer stub adapter (13–18-gauge) was tied into the trachea of the rodents, while an endotracheal tube (3–4-mm-ID) was tied into the rabbit trachea. The tracheostomies showed no apparent leaks and all tracheal tubes were mated with the special respiratory valve described below. Measurements were made on animals in the supine position.

Respiratory valve. We modified a three-way stopcock (BD 3138) to be used as a small-animal respiratory valve (Fig. 1). An 18-gauge stainless steel tube was silver soldered into a port drilled through one side of the hub of the male Luer fitting, and this tube was connected with about 10 cm of PE-160 tubing to a differential pressure transducer (MP 45, ± 50 cmH_2O, Validyne Engineering, Northridge, CA) to measure airway pressure. The transducer was calibrated with a water manometer. With the valve connected to the tracheostomy tube, airway pressure was monitored continuously and displayed on a strip chart recorder. All airway pressures were measured and are reported relative to atmospheric pressure.

Measurement of Lung Volumes and Diffusing Capacity

The terms TLC and RV are used in this study with anesthetized animals referring to the gas volumes in the lungs at airway pressures of +25 cmH_2O (TLC) and at −10 cmH_2O (RV). At an airway pressure of 25 cmH_2O the lungs are on the flat portion of the pressure-volume curve. To confirm that an airway pressure of 10 cmH_2O less than atmospheric pressure was sufficient to deflate the lungs to RV, we used six normal animals and measured the vital capacity (VC) from different airway pressures. The VC was constant in each animal at airway pressures from −5 cmH_2O to −20 cmH_2O.

FRC has been defined in this study as the resting lung volume attained during the apnea that followed a brief period of hyperventilation. The factors that set FRC, and to some extent RV, are different in anesthetized vs. normal awake animals. We have attempted to use a constant level of anesthesia so that comparisons can be

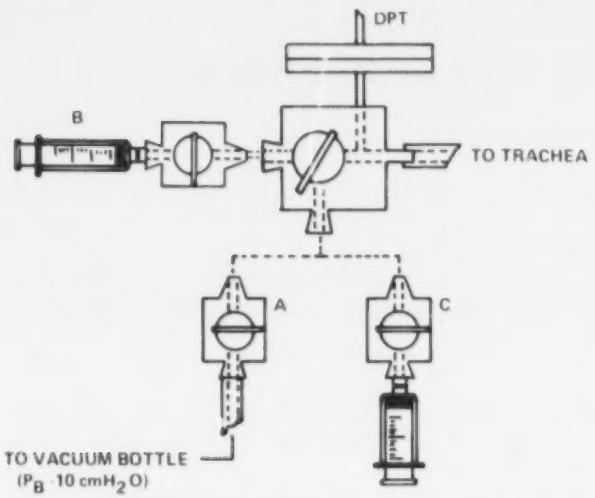

FIG. 1. Diagram of respiratory valve used to measure lung volumes and diffusing capacity of small laboratory animals. DPT, differential pressure transducer; A, connection to pressure reservoir; B, syringe to inject test gas and to collect dead-space gas; C, syringe to collect end-expiratory sample.

made within this study and to other animals studied at a similar level of anesthesia.

A specific measurement sequence was always used. VC and inspiratory capacity (IC) were each determined three times and then TLC and D_{LCO} were alternately measured three times each. The average value of the three measurements is reported.

Vital capacity. The lungs were deflated to RV by connecting one port of the respiratory valve to a 4-liter reservoir at −10 cmH_2O; the valve was then turned, and the lungs were inflated from a syringe to TLC. The volume necessary to inflate the lungs from −10 to 25 cmH_2O was read from the syringe and was recorded as the VC.

Inspiratory capacity. The animals were ventilated for 5–20 s on a Harvard small-animal respirator with peak inspiratory pressures of 12 cmH_2O at a rate of about 10–15 cycles/min faster than the spontaneous breathing frequency. During apnea the animal was connected to the respiratory valve and the lungs were again inflated from a syringe to TLC. The volume necessary to inflate the lungs from FRC to 25 cmH_2O was recorded as the IC. No correction was made for gas compression when the volume for VC or IC was read from the syringe.

Total lung capacity. A test gas containing 0.5% neon (Ne) and equivalent in volume to the VC was injected into and out of the lungs 10 times from RV. The final Ne concentration in this sample was analyzed and TLC was calculated on the basis of the Ne dilution (1).

Single-breath diffusing capacity of the lungs for carbon monoxide. The animals were again hyperventilated, and during the period of apnea the lungs were inflated from FRC through the respiratory valve with a volume of test gas equivalent to the IC and which contained 0.5% Ne and 0.5% carbon monoxide (CO). Lung inflation was maintained for approximately 8 s and then a volume equivalent to 50% of the IC was withdrawn into the syringe to wash out the dead-space gas; the respiratory valve was turned, and an approximately equal volume, used as a mean end-expiratory sample, was removed into a second syringe. For some studies D_{LCO} was measured from RV. In this case, one port of the respiratory valve was connected to the reservoir (maintained at −10 cmH_2O pressure to deflate the lung to RV). The valve was turned and a volume of the test gas equivalent to the VC was injected. During the breath-holding maneuver, the reservoir was disconnected from the respiratory valve and a syringe for collection of the end-expiratory sample was reconnected. Approximately 50% of the gas was removed into the first syringe to wash out the dead-space gas; the respiratory valve was turned, and the remaining gas was collected in the second syringe as an end-expiratory sample. The exact time of the breath holding was measured from the recorded airway pressure (Fig. 2). The lungs were inflated or deflated as rapidly as possible but care was taken not to generate excessive airway pressures.

Gas analysis. Ne and CO concentrations were measured on a gas chromatograph for respiratory gases (model AGC 111, Carle Instruments, Fullerton, CA). Carbon dioxide and water vapor were separated on Porapak QS (100 mesh, 3 ft x ⅛ in.) while neon, oxygen, nitrogen, and carbon monoxide were separated in that order on a molecular sieve 13 × (100 mesh, 3 ft x ⅛ in.). The minimum sample volume for the reproducible measurements was 0.6 ml. Disposable plastic syringes with metal stopcocks were used to collect the gas samples.

Calculations. FRC and RV were derived arithmetically. The apparent alveolar volume (V_A) and the D_{LCO} were calculated using standard formulas (see APPENDIX and Refs. 1, 4). The gas concentrations measured in the TLC samples and in the end-expiratory samples were corrected to account for the dilution of these samples by the dead space in the respiratory valve plus the syringe and stopcock. We corrected for a dead-space volume of 0.15 ml. These corrections were especially important in measurements made on the smaller animals when the sample volume collected may be as small as 0.9 ml. Barometric pressure was measured each day with a mercury barometer.

By convention, lung volumes are reported at BTPS. Body temperature falls when these animals are anesthetized with pentobarbital sodium, so we measured the body temperature in four animals of each species and used the value that occurred from 20 to 30 min of anesthesia to derive the appropriate correction factor.

Several factors that might affect the measurement of lung volumes and the D_{LCO} have been evaluated in rats. The results are compared when measurements are made in different body positions, from FRC or RV, to different airway pressures, following hyperventilation, and in male vs. female rats.

Lung weight. At the end of the study the abdomen was opened and the abdominal vessels cut. After 30–60 s the lungs were removed, dissected free at the hilum, and weighed.

Statistics. Regression analysis was used to examine the relationship between a specific pulmonary function measurement, Y, and body weight, X. Animal species

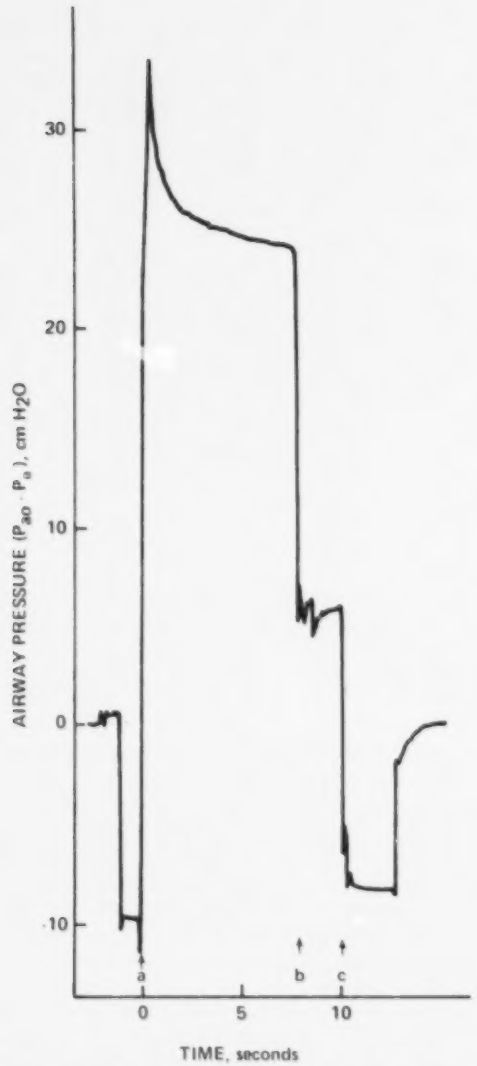

FIG. 2. Airway pressure during measurement of diffusing capacity in small animals. *Arrows* indicate following: a, injection of test gas into airway; b, withdrawal of approximately 50% of test gas; c, withdrawal of final 50% of test gas. Time of breath holding is measured from a to c. This tracing was made when D_{LCO} was measured from residual volume ($P_{ao} - P_B = -10$ cmH_2O). Initial peak in pressure is in part due to gas compression on injection at airway opening. Gradual decline in pressure over next 8 s is probably related to stress relaxation in lung. Pao, pressure at airway opening.

specific regression models of the form $Y = \alpha X^{\beta}$ were fit using simple linear regression techniques, after transforming X and Y to their common logarithms. An estimate of the experimental error variance was obtained for each variable and, whenever possible, the sampling assay error was also determined. Multivariate analyses of variance were used to analyze the data obtained in the experiments that examined the effects on D_{LCO} of the factors summarized in Table 3 (e.g., hyperventilation, airway pressure, and body position). Analysis of covariance using body weight as a covariate was used to analyze for differences between measurements of D_{LCO} from RV as compared to FRC and between male and female rats. Information on the error variance and technique variance are presented to assist those who may wish to plan research protocols using these techniques.

RESULTS AND DISCUSSION

We have described techniques that can be used to measure the D_{LCO} and lung volumes in small laboratory animals and have presented a summary of the data from measurements made in anesthetized hamsters, rats, guinea pigs, and rabbits. Regression analysis of the lung weights, lung volumes, and the D_{LCO} vs. body weight are summarized in Table 1. This data can be used to predict a value for any of these measurements if the body weight of an animal is known.

Lung Volumes

The data points and the calculated regression lines for the relationships between lung volumes and body weight of each species are given in Fig. 3. We have depicted the data that is derived from the direct measurements, TLC, VC, and IC (Fig. 3, upper panel) and have shown the derived values, FRC and RV (Fig. 3, lower panel). The models account for most of the variation present in the data, as indicated by the large r^2 values given in Table 1; the fitted lines are all statistically significant ($P < 0.05$).

The lung volumes have all been converted to BTPS. We found the body temperatures to be 38.6 ± 0.1, 36.6 ± 0.5, 35.6 ± 0.5, and 31.4 ± 1.3°C (mean ± SD) for the anesthetized rabbits, guinea pigs, rats, and hamsters, respectively; the appropriate correction factor to convert ATP (at 22°C) to BTPS for each of these species is 1.10, 1.08, 1.08, and 1.05, respectively.

Gas dilution techniques have been used by other investigators to measure FRC in small animals. King (3) used a closed-circuit nitrogen rebreathing method to estimate FRC in rats of different sizes. The slope of his regression line is not statistically different from ours; however, the data from the current study are less variable ($r^2 = 0.96$ vs. $r^2 = 0.58$). Johanson and Pierce (2) reported the FRC of 4-mo-old rats (338 g) as 11% less than the FRC that we would estimate (3.3 vs. 3.7 ml). At 18 mo (493 g) the FRC they measured was 33% larger than our estimate (6.1 vs. 4.6 ml). Snider and his co-workers (6) measured IC and FRC in hamsters by Ne dilution and plethysmographically. Their volumes for IC were larger than we would estimate for hamsters of this size (4.5 ml and 4.8 ml vs. 4.1 ml). They reported an FRC of 1.2 ml when measured by Ne dilution, which is about 40% of the volume we would estimate. The FRC they estimated plethysmographically was about 2.8 ml, which is comparable to the value that we would estimate for hamsters of this size (3.0 ml).

It is likely that in the absence of anesthesia, FRC is set by factors that include a higher tidal volume and frequency coupled with expiratory braking (persistent inspiratory muscle activity or increased glottal resistance) as has been reported in mice by Vinegar and his colleagues (8).

TABLE 1. *Summaries of regression analyses on logarithmic scale*

		LW, g	TLC, ml	RV, ml	FRC, ml	VC, ml	IC, ml	D_{LCO}, ml·min^{-1}·Torr^{-1}
Hamster (n = 15)	Intercept	−1.24	−1.10	−2.28	−2.23	−0.96	−0.92	−2.83
	Slope	0.48	0.93	1.27	1.29	0.78	0.73	0.91
	r^2	0.861	0.946	0.923	0.938	0.890	0.884	0.909
	$S_{Y.X}$	0.0337	0.0389	0.0644	0.0581	0.0479	0.0463	0.0503
	S_T		0.0214	0.0748	0.0582			0.0424
Rat (n = 21)	Intercept	−1.42	−0.55	−1.02	−0.82	−0.72	−0.84	−2.60
	Slope	0.65	0.65	0.58	0.55	0.67	0.69	0.75
	r^2	0.946	0.973	0.936	0.958	0.962	0.960	0.955
	$S_{Y.X}$	0.0495	0.0340	0.0488	0.0366	0.0419	0.0450	0.0524
	S_T		0.0129	0.0505	0.0374			0.0281
Guinea pig (n = 12)	Intercept	−1.04	−0.31	−0.82	−0.26	−0.46	−0.85	−2.56
	Slope	0.53	0.59	0.53	0.42	0.60	0.70	0.74
	r^2	0.925	0.960	0.880	0.883	0.936	0.932	0.924
	$S_{Y.X}$	0.0374	0.0293	0.0479	0.0373	0.0386	0.0468	0.0523
	S_T		0.0092	0.0423	0.0229			0.0280
Rabbit (n = 14)	Intercept	−0.43	−0.54	−1.23	−0.50	−0.62	−1.02	−3.15
	Slope	0.39	0.74	0.76	0.60	0.72	0.82	0.92
	r^2	0.617	0.780	0.468	0.525	0.782	0.785	0.872
	$S_{Y.X}$	0.0450	0.0567	0.1174	0.0823	0.0552	0.0619	0.0512
	S_T		0.0252	0.0982	0.0623			0.0272
Combined species (n = 62)	Intercept	−1.75	−0.97	−1.48	−1.45	−1.13	−1.15	−2.55
	Slope	0.79	0.85	0.81	0.87	0.86	0.84	0.74
	r^2	0.972	0.981	0.944	0.958	0.982	0.981	0.983
	$S_{Y.X}$	0.0785	0.0696	0.1161	0.1060	0.0678	0.0680	0.0471

Intercept and slope from regression: $\log_{10} Y = b_0 + b_1 \log_{10}$ (body wt) where Y = TLC, RV, etc. r^2, Proportion of total variation about the mean response explained by the regression model; $S_{Y.X}$, standard deviation about the regression line; S_T, standard deviation of the measurement technique associated with the triplicate assay; n, number of animals; LW, lung weight; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; VC, vital capacity; IC, inspiratory capacity; D_{LCO}, diffusing capacity for carbon monoxide. All lung volumes are in BTPS.

Diffusing Capacity

A comparison of D_{LCO} vs. body weight is presented for each species in Fig. 4. Other investigators have measured the diffusing capacity for CO in small animals using rebreathing techniques. Turek et al. (7) measured a D_{LCO} of 0.084 ml·min^{-1}·Torr^{-1} in 203-g rats using a steady-state method requiring the collection of an arterial blood sample. Their value is 38% less than the value we would predict for a rat this size. Johanson and Pierce (2) used a rebreathing technique and measured the apparent diffusing capacity in rats weighing 338 g (4 mo old) and 493 g (18 mo old). The D_{LCO} they reported for these two groups was 0.146 and 0.241 ml·min^{-1}·Torr^{-1}. These values are 26 and 8% less than the values we would predict for rats this size. Snider and his co-workers (6) adapted these latter methods and measured the D_{LCO} in 126-g hamsters. Their value of 0.136 ml·min^{-1}·Torr^{-1} is 11% greater than that which we would estimate. These techniques are all considerably more difficult to accomplish in small animals than the single-breath techniques described here, and the calculations are more cumbersome; however, the results are comparable to those we report for animals of similar size and species.

Factors Affecting Measurements

The measurements of D_{LCO} and lung volumes in small animals is dependent on accurate techniques, the ability to estimate gas concentrations in small volumes, and a consideration of influences such as equipment dead space. Each of these areas may introduce consistent errors; however, they can each be addressed directly.

We measured the dead space in the syringes, stopcocks, and respiratory valve by filling the dead space with water and weighing the equipment before and after filling. Each sample analysis was corrected for the dilution that occurred because of this dead space. To determine if the disposable plastic syringes were permeable to CO or NE, some of the syringes were filled with 0.5% Ne and 0.5% CO in air and allowed to stand at room temperature for 24 h. There was no change in the concentration of Ne or CO after this period.

Breath-holding time. Ogilvie et al. (4) have estimated that an appropriate breath-holding time to measure the single-breath diffusing capacity in humans is 10 s. Robinson and his colleagues (5) used 10 s when they studied single-breath D_{LCO} in beagle dogs. When the lungs are inflated initially, transient changes in pulmonary blood volume and cardiac output may occur and could change the measured D_{LCO} for several seconds. We therefore measured the D_{LCO} in rats and used breath-holding times of 5, 10, and 15 s. There was a fall in the estimated D_{LCO} as we increased the time of breath holding (Table 2, *A*). The reason for this fall in D_{LCO} is unclear; however, since 10 s has been used by other investigators, and it is a convenient time period in which to do the measurement, we decided to use 10-s breath holding routinely. It appears that the time of breath holding is important to this measurement and care should be exercised to keep the period of breath holding as close to 10 s as possible. A careful worker can reproduce this period of breath holding within 0.25 s.

The Hering-Breuer reflex is strong in all of the species we studied and there was rarely any inspiratory effort by

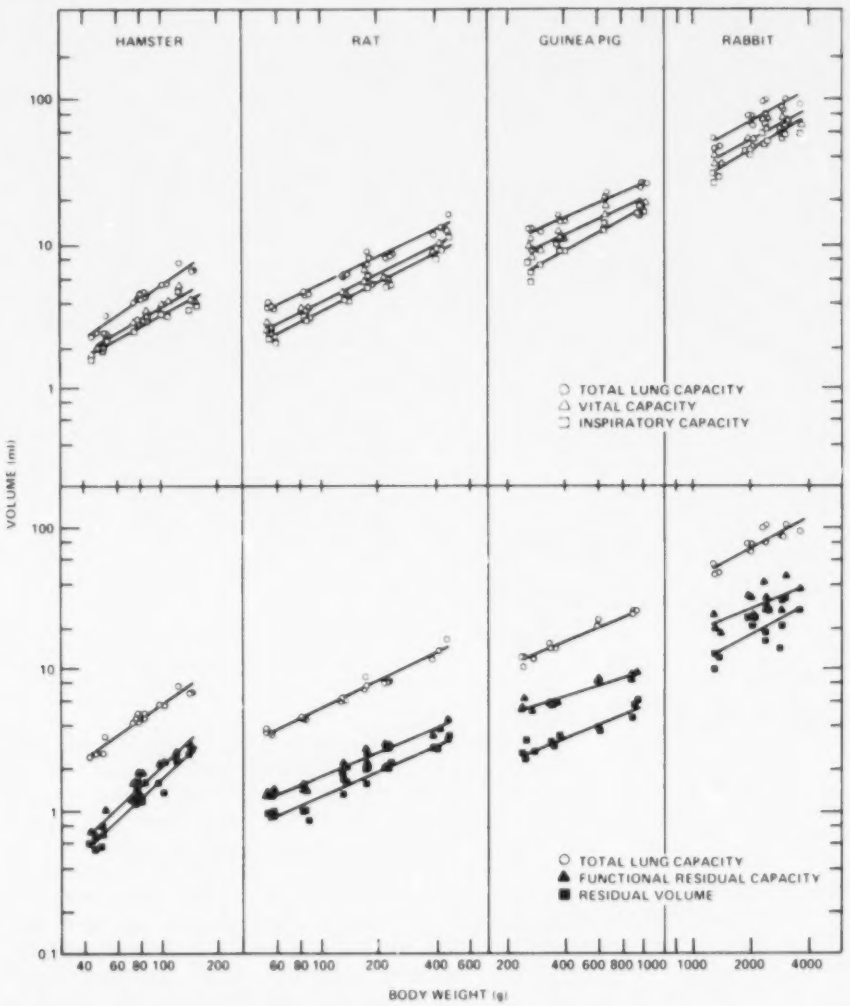

FIG. 3. Relationships between lung volumes or capacities and body weight of hamsters, rats, guinea pigs, and rabbits. *Upper panel, open symbols* illustrate data from original measurements (total lung capacity, vital capacity, and inspiratory capacity). *Lower panel, closed symbols* represent data for derived values (functional residual capacity and residual volume).

an animal once we began to inflate the lungs.

Injection volume. We also measured D_{LCO} in rats by injecting different volumes of the test gas. In one study, D_{LCO} was estimated from FRC to airway pressures of 20 and of 30 cmH_2O. The apparent V_A increased significantly from 9.4 to 13.3 ml ($P = 0.029$) with the increased airway pressure; however, there was no change in the D_{LCO} (Table 2, *B*).

In a separate study, D_{LCO} was measured in rats after injecting an appropriate volume from FRC or RV so that the lungs were inflated to TLC. The apparent V_A was not different when measured from FRC or RV (11.2 and 11.1 ml, respectively, $P = 0.3$). Likewise, D_{LCO} was not significantly different when compared in these two measurements (Table 2, *C*).

Other factors. To determine if there was significant retention and back diffusion of CO from the blood into the alveoli (CO back pressure) following estimation of the TLC or the D_{LCO}, we made three measurements of D_{LCO} at 1-min intervals in eight different rats. The first measurement of D_{LCO} was not different from any of the subsequent measurements (Table 2, *D*), and we presume that CO does not accumulate in the blood in such a manner as to affect subsequent measurements of D_{LCO}.

Because FRC was estimated following a period of hyperventilation, D_{LCO} was measured from RV three times in 10 animals. Subsequently each animal was hyperventilated in a manner similar to that used to produce apnea in FRC, and then D_{LCO} was again measured from RV. This maneuver was repeated three times. There was no difference in the D_{LCO} we measured before or after the hyperventilation (Table 2, *E*), and we concluded that this maneuver did not cause pulmonary or cardiovascular changes affecting the measurement of D_{LCO}.

Ten rats were studied in four different body positions: left and right lateral decubitus, supine, and prone. There was no difference in the D_{LCO} measured in these animals (Table 2, *F*), and we assume that the mechanisms affecting the estimation of D_{LCO} in larger animals in different body positions do not influence this measurement in these small animals.

We also measured the D_{LCO} in five rats using 0.5 and 1.0% CO. When D_{LCO} was measured using 1.0% CO, the value was about 6% greater than that found using 0.5%

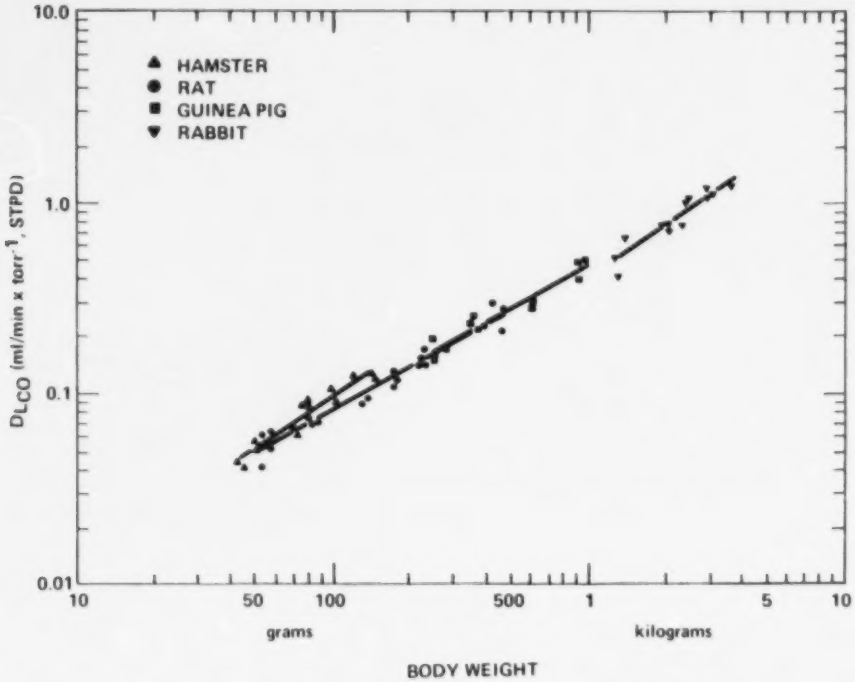

FIG. 4. Diffusing capacity for carbon monoxide (D_{LCO}) vs. body weight of small animals.

TABLE 2. *Factors affecting measurement of* D_{LCO}

	D_{LCO}, ml·min^{-1}·Torr^{-1}	*P*	Body Wt, g	*n*
A. Breath-holding time, s				
5	0.251			
10	0.219	0.001	328 ± 36	6
15	0.186			
B. Different airway pressures, cmH_2O				
20	0.184	0.95	249 ± 18	6
30	0.183			
C. From FRC or RV				
FRC	0.175	0.15	272 ± 3	9
RV	0.204			
D. Duplicate estimates at 1-min intervals				
1	0.138			
2	0.136	0.55	180 ± 4	8
3	0.136			
E. Hyperventilation				
Spontaneous ventilation	0.187	0.97	271 ± 8	10
Hyperventilation	0.186			
F. Body position				
Supine	0.160			
Prone	0.164	0.95	217 ± 2	10
Left side	0.160			
Right side	0.165			
G. Initial CO concn, %				
0.5	0.048	0.028	64 ± 1	5
1.0	0.051			
	$F_{A_{Ne}}/F_{E_{Ne}}$ × 100	*P*	Body Wt, g	*n*
H. Neon concn in end-expiratory samples of different size, % of VC				
18	89.7*			
33	94.2	0.0003	200 ± 6	6
50	94.7			
75	96.5*			

All data in this table are normalized to the mean body wt for each study. *P*, probability of no significant difference; body wt (mean ± SE); *n*, no. of animals. * These values differ from the overall mean.

CO (Table 2, *G*).

The relative size of the end-expiratory sample was varied from 18 to 75% of the expired volume. The Ne concentrations in the largest end-expiratory sample (75%), which may contain relatively more dead-space gas, and in the smallest end-expiratory sample (18%), which may contain gas from more compliant portions of the lung, were significantly different from the mean and changed in a predictable manner. However, over the midrange (at 33 and 50%) there was no difference in the Ne concentration in the end-expiratory samples (Table 2, *H*). Analysis of an end-expiratory sample, which is about 50% of the expired volume, should provide a reproducible mean value for the alveolar gas concentration.

The determination of the apparent V_A and the TLC is dependent on mixing of the Ne in the test gas with the gas in the lung. The estimate of V_A is part of the single-breath maneuver and will measure only the gas volume in portions of the lungs that readily communicate with the airways. Ten seconds is adequate time for the concentration of the test gas to become uniform by diffusion; therefore, the measurement of V_A should be adequate in normal animals. Likewise, when TLC is determined, the test gas is washed into and out of the lungs repeatedly to ensure complete mixing of the gas in all areas of the lung. To determine that 10 cycles are adequate to ensure complete mixing, we have used as few as 5 and as many as 20 cycles. There was no difference in the TLC that we estimated.

Our measurements have all been made in healthy animals with no evidence of lung disease. If studies are projected in animals with pulmonary disease, especially diseases affecting distribution of ventilation, it may be necessary to reevaluate the measurement of V_A and TLC. For instance, if areas of the lung exist that do not com-

municate adequately with the airways (e.g., bullae) then these gas dilution techniques will underestimate TLC. However, the measurement of the apparent V_A in such diseased animals should be related to the estimate of $D_{L_{CO}}$. That is, the diffusion of the test gases can only occur from ventilated areas, and these are the portions of the lung that will participate in the estimate of V_A and $D_{L_{CO}}$.

Lung volumes and $D_{L_{CO}}$ were measured in 21 male and 23 female rats to see if there were differences related to the sex of the animal. These data were normalized to the mean body weight of the combined group using analysis of covariance. The estimates of TLC and VC for female rats were 13 and 16% greater than for males, respectively. There were, however, no differences in the RV or the $D_{L_{CO}}$ between the groups of male and female rats (Table 3).

Table 1 gives the standard deviations of the technique and experimental errors. By using these error estimates, it can be determined whether refinements in the technique are critical for reducing the experimental error. Estimating the proper sample size in an experiment requires power function calculations that control the error associated with rejecting a true hypothesis (type I error), as well as with accepting a false hypothesis (type II error). For these calculations the minimum detectable change and an estimate of the experimental error per animal are required. The best estimate of the experimental error variance for each of the measurements made in this study can be obtained by squaring the value of $s_{Y \cdot X}$ given in Table 1.

Scaling

These data provide useful information for the scaling of the diffusing capacity and lung volumes. For example, if we use representative values for the physiologically measured diffusing capacity of small animals (present study), cat (9), dog (5), and man (4), the overall slope for $D_{L_{CO}}$ vs. body weight is about 0.98. This slope compares favorably with the slope for diffusing capacity of the lungs for oxygen that Weibel (10) has calculated for several species on the basis of morphometric considerations. The slope he calculated is 0.96. Our individual slopes for each species are less than the overall slope because, in part, they reflect aging effects within individual species.

TABLE 3. *Physiological variables in male and female rats*

	Body Wt, g	Lung Wt, g	TLC, ml	VC, ml	RV, ml	$D_{L_{CO}}$, ml·min^{-1}·Torr^{-1}
Male (n = 21)	128	1.18	9.2	7.5	1.6	0.146
Female (n = 23)	186	1.11	10.4*	8.9*	1.4	0.151

Lung volumes in BTPS, pulmonary diffusing capacity for carbon monoxide given in $D_{L_{CO}}$ in STPD. Data are detransformed adjusted means from analysis of covariance using body weight as a covariate. Statistical analyses were performed using the logarithmically transformed data. n, no. of animals. * The male and female means are significantly different ($P < 0.05$).

The methods we have described are basically adaptations of methods that have been used to make similar measurements in man. They lend themselves to modification, and other investigators may find it advantageous to institute some changes. For instance, if $D_{L_{CO}}$ is to be measured on a routine basis, it is easier and faster to make the measurement from RV rather than FRC. For use with small laboratory animals, special manipulations and precautions have been taken to adapt the rather straightforward assumptions on which these tests are based. It is possible, with attention to such details as gas analysis in small samples and additional corrections for equipment dead space, to make similar measurements in even smaller animals.

APPENDIX

Equations Used to Calculate Results

$$TLC = VC \frac{[Ne]_{initial}}{[Ne]_{final}} \quad (1)$$

$$FRC = TLC - IC \quad (2)$$

$$RV = TLC - VC \quad (3)$$

$$F_{A_{CO_0}} = \frac{(F_{I_{CO}}) \times (F_{A_{Ne}})}{F_{I_{Ne}}} \quad (4)$$

$$V_{A(ATPS)} = \frac{(F_{I_{Ne}}) \times (VC)}{F_{A_{Ne}}} \quad (5)$$

$$V_{A(STPD)} = V_{A(ATPS)} \frac{(P_B - P_{H_2O})(273)}{(760)(273 + °C)} \quad (6)$$

$$D_{L_{CO}(STPD)} = \frac{60 \times V_{A(STPD)}}{(P_B - P_{H_2O}) \times t} \times \ln \frac{F_{A_{CO_0}}}{F_{A_{CO_t}}} \quad (7)$$

Equation 1 is derived by substituting *Eq. 3* in the equation used to calculate RV by closed-circuit gas dilution (1).

Glossary

TLC	total lung capacity
VC	vital capacity
$[Ne]_{initial}$	initial concentration of Ne
$[Ne]_{final}$	final concentration of Ne
FRC	functional residual capacity
IC	inspiratory capacity
$F_{A_{CO}}$	fractional concentration of alveolar CO
$F_{I_{CO}}$	fractional inspired concentration of CO
$F_{A_{Ne}}$	fractional concentration of alveolar Ne
$F_{I_{Ne}}$	fractional inspired concentration of Ne
V_A	alveolar volume
ATPS	ambient temperature and pressure, saturated with water vapor
STPD	standard temperature and pressure, dry
P_B	partial barometric pressure
P_{H_2O}	partial water pressure
$D_{L_{CO}}$	pulmonary diffusing capacity for carbon monoxide
t	time
$F_{A_{CO_0}}$	fractional concentration of alveolar CO at time zero
$F_{A_{CO_t}}$	fractional concentration of alveolar CO at t

The authors thank Drs. Phillip Bromberg and Mitch Freedman who discussed this research and made helpful suggestions.

Address reprint requests to: J. J. O'Neil, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, NC 27711.

Present address of J. Takezawa: Department of Anesthesiology, Osaka University Medical School, Osaka, Japan.

Received 13 April 1979; accepted in final form 13 February 1980.

REFERENCES

1. COMROE, J. H., JR., R. E. FORSTER II, A. B. DUBOIS, W. A. BRISCOE, AND E. CARLSEN. *The Lung, Clinical Physiology and Pulmonary Function Tests*. Chicago, IL: Year Book, 1962.
2. JOHANSON, W. G., JR., AND A. K. PIERCE. Lung structure and function with age in normal rats and rats with papain emphysema. *J. Clin. Invest.* 52: 2921–2927, 1973.
3. KING, T. K. C. Measurement of functional residual capacity in the rat. *J. Appl. Physiol.* 21: 233–236, 1966.
4. OGILVIE, C. M., R. E. FORSTER, W. S. BLAKEMORE, AND J. W. MORTON. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J. Clin. Invest.* 36: 1–17, 1957.
5. ROBINSON, N. E., J. R. GILLESPIE, J. D. BERRY, AND A. SIMPSON. Lung compliance, lung volumes, and single-breath diffusing capacity in dogs. *J. Appl Physiol.* 33: 808–812, 1972.
6. SNIDER, G. L., B. R. CELLI, R. H. GOLDSTEIN, J. J. O'BRIEN, AND E. C. LUCEY. Chronic interstitial pulmonary fibrosis produced in hamsters by endotracheal bleomycin. *Am. Rev. Respir. Dis.* 117: 289–297, 1978.
7. TUREK, Z., M. GRANDTNER, B. E. M. RINGNALDA, AND F. KREUZER. Hypoxic pulmonary steady-state diffusing capacity of CO and cardiac output in rats born at a stimulated altitude of 3500 m. *Pfluegers Arch.* 340: 11–18, 1973.
8. VINEGAR, A., E. E. SINNETT, AND D. E. LEITH. Dynamic mechanisms determine functional residual capacity in mice, *Mus musculus*. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 46: 867–871, 1979.
9. WATANABE, S., AND R. FRANK. Lung volumes, mechanics, and single-breath diffusing capacity in anesthetized cats. *J. Appl. Physiol.* 38: 1148–1152, 1975.
10. WEIBEL, E. R. Morphometric estimation of pulmonary diffusion capacity. *Respir. Physiol.* 14: 26–43, 1972.